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Supporting Information

Significantly Enhanced DNA Thermal Stability Resulting from Porphyrin H-Aggregate Formation in the Minor Groove of the Duplex

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Materials Unless otherwise stated, reagents and solvents were purchased from Merck and used without further purification. $Ni(OAc)_2 \cdot 4H_2O$ was purchased from Acros and MeOH and $CuSO_4 \cdot 5H_2O$ from Scharlan. Lithium perchlorate was obtained from Aldrich and $MgSO_4$ from BDH. 2′-*O*-Propargyl uridine and 2′-*O*-Propargyl adenosine phosphoramidites are commercially available from ChemGenes Corporation. Chromatographic purification of compounds was performed using Silica gel 60 (230-400 mesh, SDS). ^{1}H NMR spectra were recorded on a 400 MHz Brüker instrument using Topspin software. Chemical Shifts are relative to the residual DMSO protium at 2.51 ppm. HR-ESI mass spectroscopic data for the porphyrins was obtained using a Waters LCT ESI-TOF Mass spectrophotometer in the negative mode in DCM/MeOH. IR spectra were recorded on a Nicolet 5700 FT-IR from Thermo electron corporation using an ATR attachment. Thin-layer chromatography (TLC) analyses were carried out with TLC plates 60 F254 purchased from Merck and were visualized in UV light (254 nm) when necessary. Unmodified oligonucleotides **ON1** and **ON2** were purchased from IDT (USA).

Synthesis of A

4-[*trans*-2-(5,10,15,20-Tetraphenylporphyrin-2-yl)ethen-1-yl]azidomethylbenzene

DBU (250 μL, 1.67 mmol, 3.1 eq) was added to a solution of 5,10,15,20-tetraphenylporphyrin phosphonium salt[1, 2] (500 mg, 0.54 mmol) and 4-(azidomethyl)benzaldehyde[3] (260 mg, 1.13 mmol, 3 eq) in DCM (100 mL) and stirred at RT under Ar. After 20 minutes TLC indicated that the phosphonium salt had completely reacted. The solvent was reduced to approximately 10 mL under vacuum, MeOH (100 mL) was added and the volume was reduced again. This process was repeated until the porphyrin precipitated. The resulting purple solid was collected by filtration as a *cis/trans* (35:65) isomeric mixture (340 mg, 76%) that also contained a small amount of TPP-CH_3 according to ^{1}H NMR. The isomeric mixture was dissolved in $CHCl_3$ (100 mL) and I_2 (103 mg, 0.41 mmol, 1.0 eq) was added. After stirring at RT overnight in the dark, a saturated aq. solution of $Na_2S_2O_3$ (100 mL) was added and stirring was continued for an additional 15 minutes. The organic layer was separated, dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to give the *trans* product. The resulting purple solid was dissolved in DCM (20 mL), diluted with

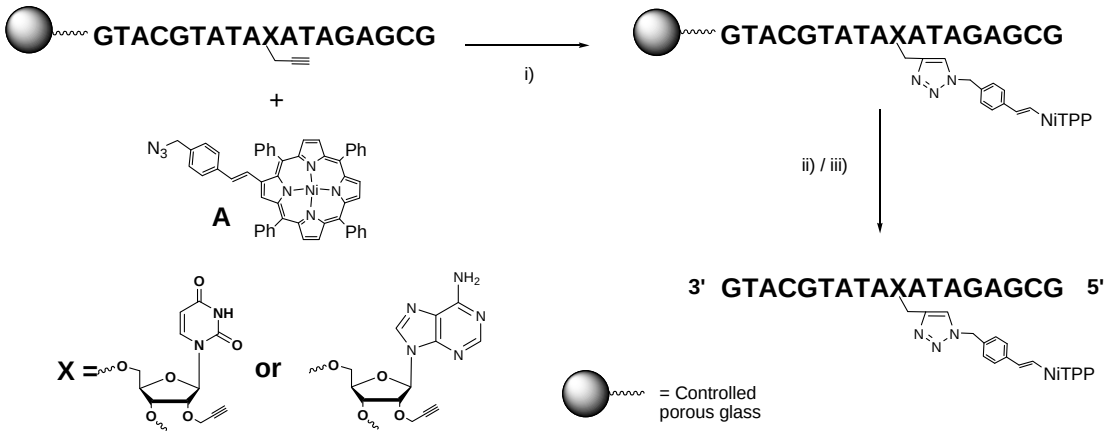
hexane (20 mL) and purified by silica gel column chromatography first eluting trace of TPP-CH_3 with DCM:hexane (1:1) then the desired product with MeOH:DCM (1:19) as a purple band. The resulting solution was reduced to approximately 10 mL under vacuum, MeOH (100 mL) was added and the volume was reduced again. This process was repeated until the porphyrin precipitated, giving pure *trans* product (270 mg, 60% overall) as a purple powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (s, 1H, $H_{\beta\text{-pyrrolic}}$), 8.83-8.72 (m, 5H, $H_{\beta\text{-pyrrolic}}$), 8.67 (d, 1H, J = 4.8 Hz, $H_{\beta\text{-pyrrolic}}$), 8.26-8.18 (m, 8H, H_{ortho}), 7.89-7.80 (m, 12H, $H_{meta,\ para}$), 7.42 (d, 1H, J = 16.2 Hz, $H_{1'\text{ or }2'}$), 7.36 (d, 2H, J = 8.1 Hz, $H_{aromatic}$), 7.30 (d, 2H, J = 8.1 Hz, $H_{aromatic}$), 6.94 (d, 1H, J = 15.9 Hz, $H_{1'\text{ or }2'}$), 4.49 (s, 2H, CH_2N_3), -2.72 (br s, 2H, NH). ESI-HRMS: Calcd for MH^+ ($C_{53}H_{38}N_7$): 772.3183, found: 772.3183. UV-Vis (CH_2Cl_2): λ_{max} [nm] ($\varepsilon \times 10^{-3}$) 423 (202), 523 (17.0), 562 (9.53), 598 (6.68), 654 (3.50) IR-ATR: (cm^{-1}) 2096.6 (azide).

4-[*trans*-2-(5,10,15,20-Tetraphenylporphyrin-2-yl)ethen-1-yl]azidomethylbenzene Nickel II (A)

$Ni(OAc)_2{\cdot}4H_2O$ (240 mg, 0.96 mmol, 12.5 eq) in MeOH (10 mL) was added to a refluxing solution of 4-[*trans*-2-(5,10,15,20-tetraphenylporphyrin-2-yl]ethene-1-yl)methylazidobenzene (60 mg, 77.8 μmol) in $CHCl_3$ (90 mL) and the reaction mixture was refluxed overnight under Ar. After cooling the solvent was reduced to approximately 10 mL under vacuum, MeOH (100 mL) was added and the volume was reduced again. This process was repeated until the porphyrin precipitated. The desired product was collected by filtration to give a red solid (60 mg, 99%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.91 (s, 1H, $H_{\beta\text{-pyrrolic}}$), 8.68-8.63 (m, 6H, $H_{\beta\text{-pyrrolic}}$), 8.03-7.95 (m, 8H, H_{ortho}), 7.85-7.73 (m, 12H, $H_{meta,\ para}$), 7.32 (d, 2H, J = 8.3 Hz, $H_{aromatic}$), 7.27 (d, 1H, J = 16.3 Hz, $H_{1'\text{ or }2'}$), 7.21 (d, 2H, J = 8.4 Hz, $H_{aromatic}$), 6.81 (d, 1H, J = 16.0 Hz, $H_{1'\text{ or }2'}$), 4.46 (s, 2H, CH_2N_3). ESI-HRMS: Calcd for M^+ ($C_{53}H_{35}N_7Ni$): 827.2302, found: 827.2312. UV-Vis (CH_2Cl_2): λ_{max} [nm] ($\varepsilon \times 10^{-3}$) 425 (176), 538 (16.1), 570 (10.2). IR-ATR: (cm^{-1}) 2096.1 (azide).

Solid-Phase Synthesis Oligonucleotides and post-synthetic click chemistry. DMT-off oligodeoxynucleotides were synthesized in a 1.0 μmol scale on 1000 Å CPG supports using MerMade 4 Automated DNA Synthesiser from BioAutomation Corporation, using 4,5-dicyanoimidazole as an activator. 2′-*O*-Propargyl uridine

phosphoramidite or 2′-*O*-propargyl adenosine phosphoramidite (8 mg) was coupled by dissolving the amidite in the activator solution (1 mL) in a plastic syringe and added to the support after the detritilation and washing cycles (coupling time of 5 minutes, Scheme 1S). After coupling, the synthesis was continued in an automatic mode. After DNA synthesis, DMT-off oligonucleotides on CPG (0.33 μmol) containing 2′-*O*-propargyl uridine or 2′-*O*-propargyl adenosine were removed from their corresponding columns and placed into a microwave reaction vessel together with compound **A** (7.67 mmol, 23 eq) in degassed DMSO (200 μL). Freshly prepared $CuSO_4 \cdot 5H_2O$ (0.32 μmol, 0.96 eq, 8 μL of a 40 mM solution in degassed H_2O) and sodium ascorbate (0.82 μmol, 2.5 eq, 25 μL of a 50 mM solution in degassed H_2O) were added. The reaction mixture was then irradiated in a microwave synthesizer (Discover, CEM Corporation, 70 °C, 100 watts, 20 min) (Scheme 1S). The content of the reaction was transferred to a microcentrifuge tube and the CPG was washed with DCM (1.5 mL). The DCM was removed and washing was repeated until the supernatant no longer showed any colour (see recovery of **A**). The red CPG was then washed with H_2O (1.5 mL) to remove any remaining inorganic salts. The obtained DMT-off oligonucleotides bound to CPG supports were treated with 32% aq NH_4OH (0.5 mL) at RT for 2 h and then at 55 °C overnight. Purification of porphyrin functionalized DMT-off ONs was accomplished using C_{18} cartridges (0.2μmol puri-pak cartridges from ChemGenes corporation), eluting oligonucleotides containing a single porphyrin with 20% CH_3CN in H_2O and then ONs with two porphyrins with 30% - 40% CH_3CN in H_2O (Figure 1). After purification ONs were freeze dried and dissolved in H_2O (100 μL, heating to 70 °C for 1 hr was required for some oligonucleotides). 0.01 M Lithium perchlorate in acetone (1.6 mL) was added to precipitate the ONs. Molecular weights of the oligonucleotides was obtained using a Bruker Daltonics Autoflex MALDI TOF in the negative mode using either 2',4',6'-trihydroxyacetophenone, 3-hydroxypicolinic acid or 6-azathiothymine as a matrix and dibasic ammonium citrate as a co-matrix (Table 1S). Oligonucleotides were desalted using C18 ziptips (Millipore) prior to loading on the MALDI plate. Purity was checked using denaturing 20% PAGE, showing a red single band with a significant retardation compared to the wild type oligonucleotide. **ON10**-**ON12** containing two porphyrins did not penetrate into the gel (Figure 1S).



Scheme 1S. Coupling reaction between porphyrin **A** and an oligonucleotide containing 2′-*O*-propargyl uridine or 2′-*O*-propargyl adenosine. Reagents and conditions: i) $CuSO_4$, sodium ascorbate, DMSO, H_2O, microwave, 20 minutes 70°C ii) 32% aq. NH_4OH iii) purification by C_{18} puri-pak column.

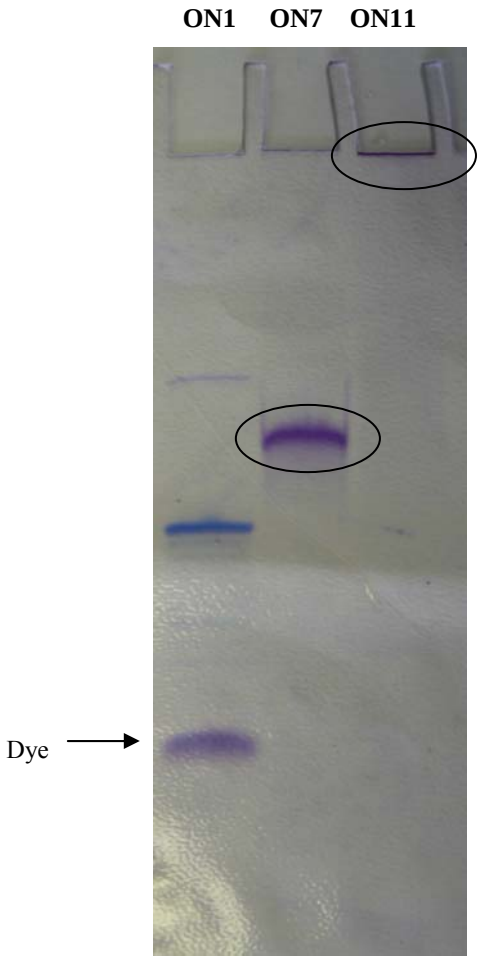


Figure 1S. Representative PAGE (20% with 7M urea) of unmodified oligonucleotide **ON1** and porphyrin modified oligonucleotides **ON7** and **ON11** captured using an Olympus digital camera after staining with Stains-All®. Porphyrin modified oligonucleotides are circled. Dyes were used for **ON1** only.

Table 1S. Oligonucleotide synthesized and their mass spectrometry analysis.

Strand	Sequence	M^+, calcd., Da	M^+, found, Da
ON1	3' GTACGTATATATAGAGCG	5562.6	----
ON2	5' CATGCATATATATCTCGC	5433.5	----
ON3	3' GTACGTATAP_UATAGAGCG	6431.2	6427.7
ON4	5' CATGCATATAP_UATCTCGC	6302.2	6297.6
ON5	5' CATGCATATP_ATATCTCGC	6316.2	6309.4
ON6	5' CATGCATAP_UATATCTCGC	6302.2	6297.0
ON7	5' CAT GCATP_ATATATCTCGC	6316.2	6316.7
ON8	5' CATGCAP_UATATATCTCGC	6302.2	6296.8
ON9	5' CATGCP_ATATATATCT CGC	6316.2	6312.8
ON10	3' GTACGTAP_UAP_UATAGAGCG	7299.8	7305.1
ON11	5' CATGCATAP_UAP_UATCTCGC	7170.8	7167.5
ON12	5' CATGCATP_ATP_ATATCTCGC	7198.8	7199.1

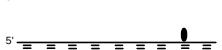
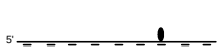
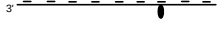
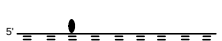
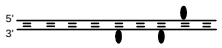
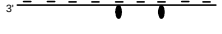
Recovery of 4-[*trans*-2-(5,10,15,20-tetraphenylporphyrin-2-yl)ethen-1-yl]methylazidobenzene Nickel II (A)

To the combined DCM washings, H_2O (50 mL) was added and the resulting solution stirred vigorously for 1 hr to remove DMSO and any remaining inorganic salts. The organic layer was extracted, dried over $MgSO_4$, filtered and the solvent was reduced to approximately 10 mL *in vacuo*. MeOH (100 mL) was added and the volume was reduced again. This process was repeated until the porphyrin precipitated. The desired product was collected by filtration to give a red solid (approximately 80-90% recovery).

Melting Temperature Measurements. Melting temperature measurements were performed on a CARY 100Bio UV-Vis spectrophotometer using a 2 × 6 Multicell block with Peltier temperature controller. Extinction coefficients for porphyrin modified oligonucleotides were calculated using the following extinction coefficients at 260 nm (L/(mol.cm)): porphyrin modified rA (21400) and porphyrin modified rU (15900) (manuscript is under preparation). The duplexes were formed by mixing the two strands each at a concentration of 1.0 μM in the appropriate buffer. The solutions were heated at 90 °C for 5 min and cooled to 10 °C for 20 min. The melting temperatures (Table 2S) were determined as the maxima of the first derivative plots of the melting curves (Figure 2S) obtained by measuring absorbance at 260 nm and 423

nm against increasing temperature (0.5 °C per min). All melting temperatures are an average of two denaturing-annealing cycles.

Table 2S. Thermal denaturing (T_d) and annealing (T_a) temperatures at 260 and 423 nm (°C)[a].

Duplex	Strands	Arrangement	$T_{d\ 260}$	$T_{a\ 260}$	$T_{d\ 423}$	$T_{a\ 423}$
1	ON1/ON2		56.0	56.0	----	----
			(46.1)[b]	(46.1)		
2	ON3/ON2		51.2	51.0	52.0	50.0
3	ON1/ON4		51.2	50.8	53.3	52.7
4	ON1/ON5		50.0	50.0	49.2	48.0
5	ON1/ON6		53.5	53.5	53.8	53.7
6	ON1/ON7		50.8	50.8	52.0	50.7
7	ON1/ON8		52.1	52.0	52.0	51.2
8	ON1/ON9		51.0	51.0	50.1	50.0
9	ON1/ON11		27.4	26.1	NVT[c]	NVT
10	ON1/ON12		25.0	25.0	NVT	NVT
11	ON3/ON4		71.8	72.6	72.2	74.5
			(57.8)	(58.0)	(58.9)	(59.8)
12	ON3/ON5		71.2	71.0	72.6	72.3
13	ON3/ON6		71.1	71.7	72.3	73.5
14	ON3/ON7		69.2	68.0	71.8	71.4
15	ON3/ON8		66.0	65.9	70.6	70.1
16	ON3/ON9		65.1	65.4	70.0	70.0
17	ON10/ON4		71.2	68.8	73.0	70.8
			(63.7)	(61.2)	(62.7)	(61.0)
18	ON10/ON6		70.8	66.2	73.2	70.0
19	ON10/ON7		71.6	69.6	71.6	70.8
20	ON10/ON8		68.0	66.2	68.5	67.6
21	ON3/ON11		69.0	66.5	70.5	69.5
22	ON3/ON12		71.1	69.8	72.0	70.8
23	ON10/ON11		>90	>90	>90	>90
			(76.2)	(74.8)	(65.8)	(65.3)
24	ON10/ON12		>90	>90	>90	>90
			(75.8)	(77.5)	(75.0)	(76.0)

[a] T_d and T_a (°C) data for antiparallel duplex melting taking from the UV-Vis melting curves (λ = 260 and 423 nm, 0.5°C/min). C = 1.0 μM of each strand in 20 mM sodium cacodylate, 100 mM NaCl and 5 mM $MgCl_2$, pH 7.2. [b] C = 1.0 μM of each strand in 20 mM sodium cacodylate, 6.25 mM NaCl, pH 7.2. [c] NVT = No visible transitions.

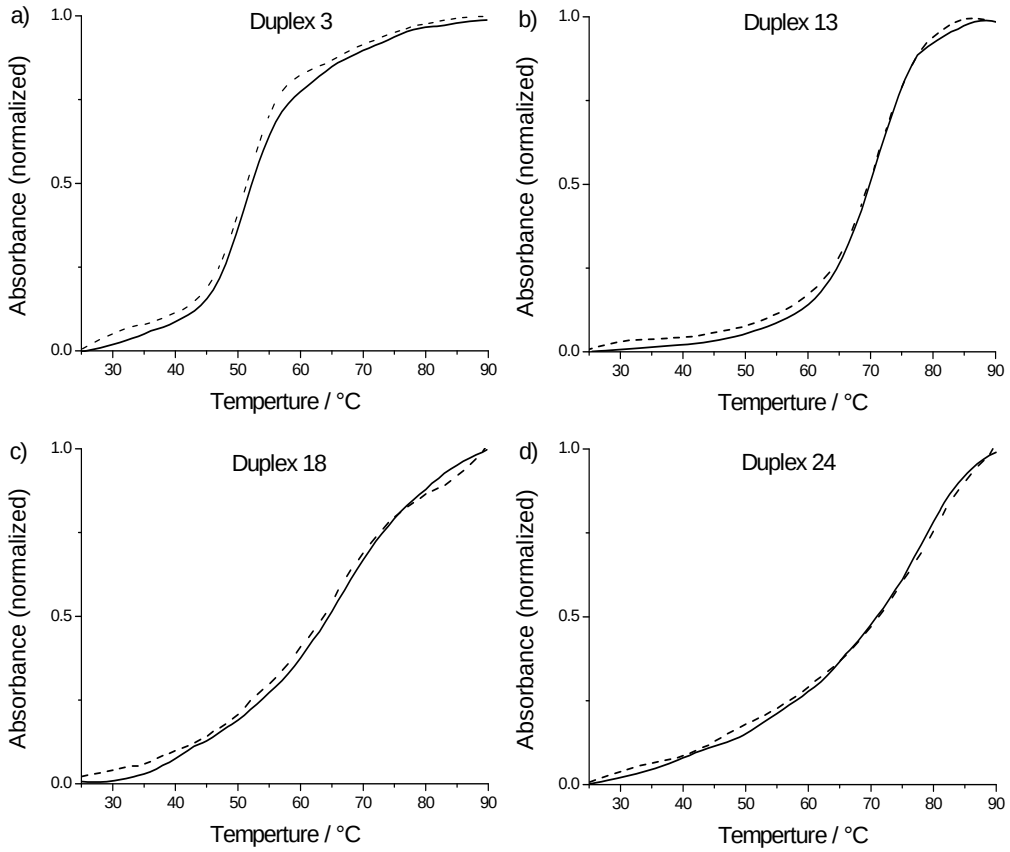


Figure 2S. Representative UV melting profiles (260 nm) of duplexes **3** (a), **13** (b), **18** (c) and **24** (d) showing both denaturing (solid) and annealing profiles (dashed). C = 1.0 μM of each strand in 20 mM sodium cacodylate, 100 mM NaCl and 5 mM $MgCl_2$, pH 7.2. for duplexes **3**, **13** and **18**. C = 1.0 μM of each strand in 20 mM sodium cacodylate, 6.25 mM NaCl, pH 7.2 for duplex **24**.

Circular Dichroism Measurements. CD spectra were recorded at 25 °C using an Applied Photophysics Chirascan CD spectrometer (150 W Xe arc) with a Quantum Northwest TC125 temperature controller. Identical solutions were used for CD spectroscopy to that used for melting studies. An average of ten scans was recorded (1 nm intervals, 240 nm/min, 1 cm pathlength), baselined against the appropriate buffer solution then smoothed (Figure 3S). Data was recorded in mdeg and converted to delta epsilon.

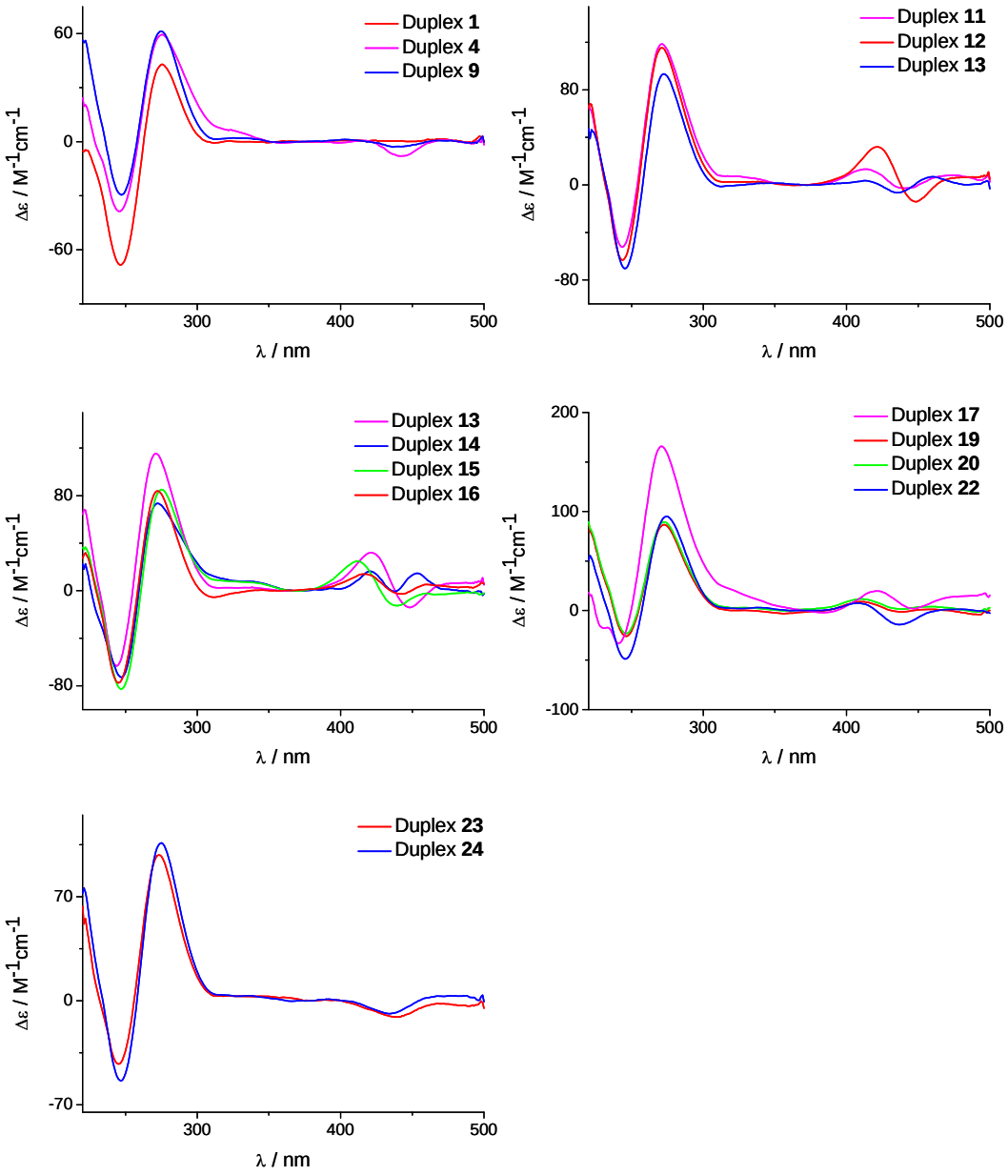
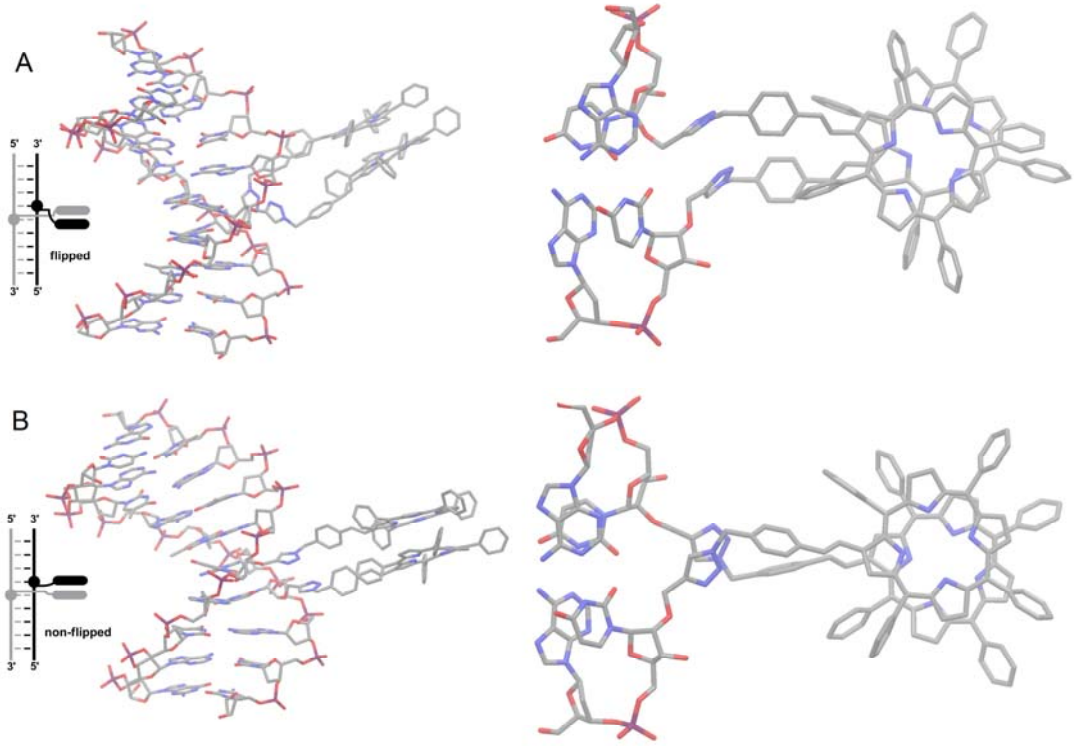


Figure 3S. CD spectra of duplexes **1**, **4**, **9** and **11-24**.

Molecular Modelling. Molecular modelling calculations and the construction of duplexes were performed using MacroModel v9.1 from Schrödinger. All calculations were conducted with AMBER* force field and the GB/SA water model.[4, 5] 11-mer duplexes containing unmetallated tetraphenylporphyrin modified uridine(adenine)-nucleotide(s) were generated from a B type DNA-DNA duplex using Maestro v9.0 from Schrödinger. Constraints ensured the planarity of the porphyrins and were based on the metal complexed porphyrin available in the Maestro software (distances: N1-N3, N2-N4; 4.132Å, force constant 100 and torsion angels: N1-C2-C3-C4, N2-C6-

C7-C8, N3-C10-C11-C12, N4-C14-C15-C16; 0.0º, force constant 100). The stochastic dynamics calculations generating 250 structures were performed using an extended cut off potential with a SHAKE algorithm to constrain bond to hydrogen. Simulation temperature was 300K, simulation time 500 ps and equilibration time 150 ps. All 250 structures were minimized using the PRCG method with convergence threshold of 0.05 KJ/mol and examined with Xcluster from Schrödinger to find representative low-energy structures (Figure 4S).



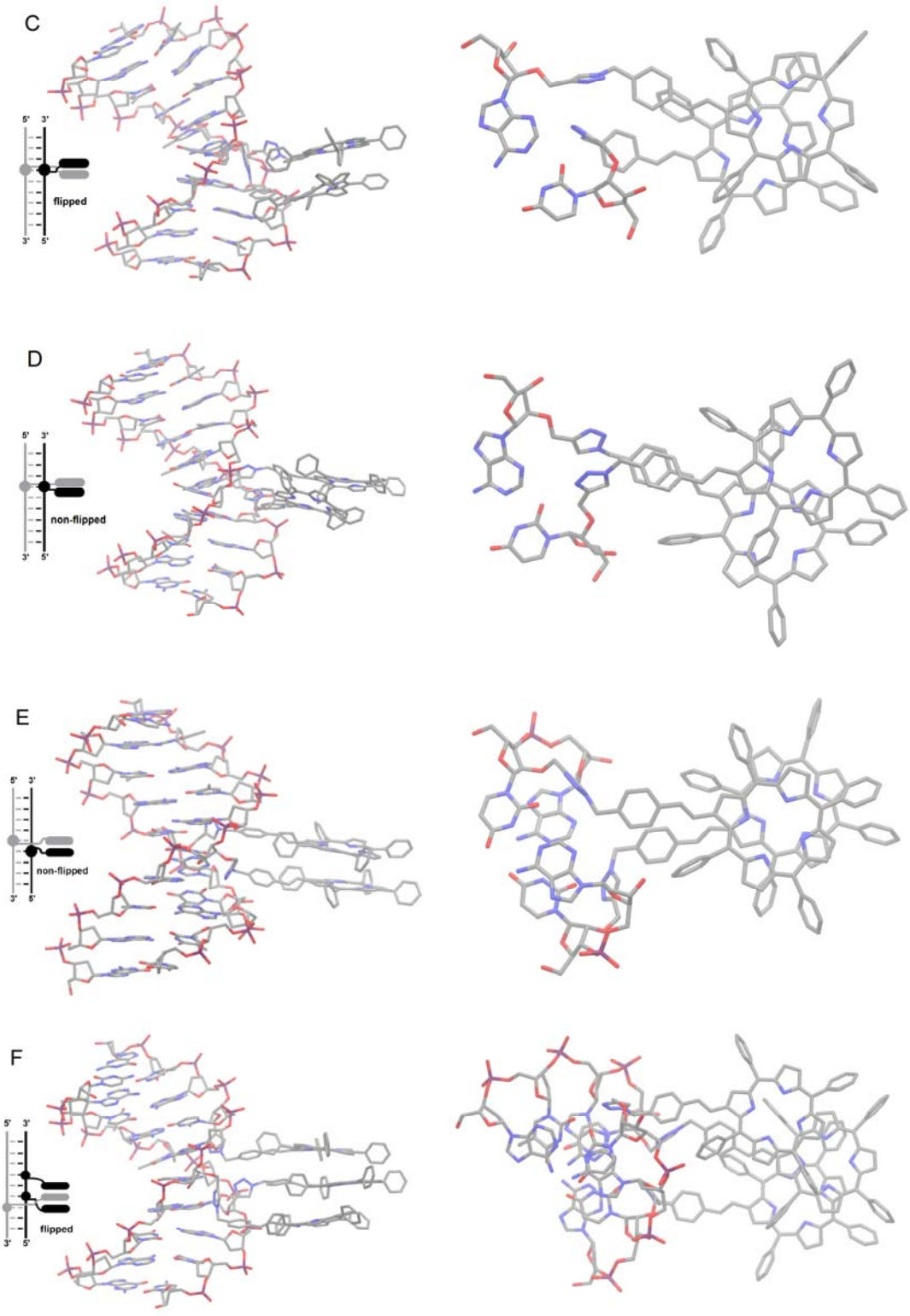


Figure 4S. An AMBER* Force-field lowest energy minimum structures of porphyrin modified duplexes. **A)** Duplex **11** with flipped porphyrins, **B)** Duplex **11** with non-flipped porphyrins, **C)** Duplex **12** with flipped porphyrins, **D)** Duplex **12** with non-flipped porphyrins, **E)** Duplex **13** with non-flipped porphyrins and F) Duplex **17** with flipped porphyrins.

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